REMARKS

I. Status of the Claims

Claims 1, 6, 9-11 and 16 and are pending in the present application. Claims 1 and 16 have been amended. No new matter has been added.

II. Telephonic Interview

The undersigned representative of the Applicants wishes to thank Examiner Humphrey, Supervising Examiner Helms and Primary Examiner Parkin for the courtesies extended during the telephonic interview conducted on October 8, 2009, with Applicant's attorney Dr. Gwen Wood and co-inventor Dr. Dean Mann. A summary of the interview follows.

Dr. Mann distinguished the claimed invention from the cited prior art. Specifically, Dr. Mann stated that none of the prior art teaches or even suggests a method of enhancing an immune response to an antigen in a mammal by administering lymphocyte conditioned medium (LCM) comprised of products generated from pooled samples of peripheral blood mononuclear cells (PBMCs) activated with anti-CD3- and anti-CD28-coated beads in combination with the antigen, in which the LCM acts by facilitating and enhancing maturation of dendritic cells (DC) and the function of antigen-presenting cells which results in an enhanced immune response to the antigen in the mammal.

Primary Examiner Parkin suggested that the claims be amended to recite this method and to send the amended claims to Examiner Humphrey and himself for their analysis and verification that none of the cited prior art teaches or suggests the claimed invention.

III. Rejection Under 35 U.S.C. § 103(a)

A. Rejection over Meidenbauer in view of Baxevanis and Quinn

On page 3 of the Office Action, claims 1, 6, 9-11 and 16 are rejected under 35 U.S.C. § 103(a) on the grounds that they are unpatentable over Meidenbauer et al. in view of Baxevanis et al. and Quinn et al. Applicants respectfully traverse the rejection.

Meidenbauer is relied upon as disclosing administering a vaccine comprising PSA in combination with an adjuvant. Baxevanis is relied upon as disclosing administering to patients cytokine-rich supernatants derived from cultures of PBMCs from healthy donors stimulated with anti-CD3 monoclonal antibodies. The Examiner acknowledges that neither Meidenbauer nor Baxevanis discloses an anti-CD28 antibody co-immobilized with an anti-CD3 antibody on beads in a PBMC culture medium for the preparation of cytokine-rich supernatants. Quinn is relied upon as disclosing co-stimulation of PBMCs with beads coated with co-immobilized anti-CD3 and anti-CD28 monoclonal antibodies. The Examiner asserts that it would have been obvious to modify the antigen-adjuvant combination of Meidenbauer by adding the supernatant of Baxevanis and to replace the anti-CD3-coated plates of Baxevanis with the anti-CD3 and anti-CD28-coated beads of Quinn. Applicants respectfully disagree.

The claimed invention is directed to enhancing an immune response in a mammal to a specific antigen by co-administering the specific antigen with products generated from pooled samples of PBMCs, referred to as lymphocyte-conditioned medium (LCM), in which the PBMCs have been activated with anti-CD3- and anti-CD28-coated beads. The LCM surprisingly acts as a potent vaccine adjuvant for the specific antigen that is administered by facilitating and enhancing dendritic cell maturation and antigen-presenting cell function, which results in enhanced immunologic responses in the mammal to the co-administered antigen.

Even if one skilled in the art would be motivated to combine the teachings of Meidenbauer with Baxevanis and Quinn, there would not be a reasonable expectation of success to achieve the claimed invention. This is because **Baxevanis solely discloses generic T cell stimulation and activation of pre-existing T cells using supernatants derived from CD3-stimulated lymphocytes but does not teach or even suggest that their supernatant can act as a potent and effective adjuvant by facilitating and enhancing an antigen-specific response when co-administered with a specific antigen**. Baxevanis states:

> Allogeneic CD3 supernatants [harvested from PBMC cultures stimulated with soluble anti-CD3] are able to activate ... **tumour-reactive lymphocytes** from cancer patient's PBMCs that lyse a **variety of tumour targets**. Abstract. [Emphasis added.]

And at page 1079, last paragraph, Baxevanis states:

> In conclusion, we demonstrate herein that cytokine-rich supernatants produced by CD3-activated HD-PBMCs during a 3-h incubation **can induce cancer patient's lymphocytes to lyse a variety of tumour targets**, including autologous tumour cells. [Emphasis added].

Thus, nowhere does Baxevanis disclose or even suggest co-administering their cytokine-rich supernatant with a specific antigen, in which the supernatant facilitates and enhances antigen specific cellular and humoral immune responses which are mediated by the action of the supernatant to induce dendritic cell maturation and antigen-presenting cell function. Baxevanis solely teaches that their cytokine-rich supernatant induces cancer patient's lymphocytes generally to lyse a variety of tumor targets, i.e., antigens generically. The teachings of Meidenbauer and Quinn do not cure the deficiency of Baxevanis to teach or suggest the claimed invention.

Various investigators have described the use of cell-free culture supernatants, referred to as "conditioned media" containing more or less well-defined mixtures of cytokines, to affect DC maturation, as discussed at page 3, 2nd full paragraph, of the specification.

For example, O' Doherty *et al.* (*J. Exp. Med.*, 178:1067-1078, 1993), teach DC maturation by culturing purified DC in monocyte-conditioned media (MCM), in culture media supplemented with purified GM-CSF and/or TNF-α, or in supernatant from PHA-stimulated PBMCs (Lymphocult®). It was shown that MCM supported DC viability and characteristic morphology better than the two purified cytokines (either alone or in combination) or in Lymphocult.®

Kato *et al.* (*J. Leukocyte Biology*, 70:941-949, 2001) teach DC maturation by using T-cell conditioned media (TCCM) containing the cytokines CD40L, TNF-α and IFN-γ prepared from cell-free supernatants of anti-CD3-activated T cells, which was shown to be more effective than adding a combination of the cytokines or the addition of MCM.

Reddy *et al.* (*Blood*, 90:3640-3646, 1997) teach DC maturation by first exposing T cell-depleted mononuclear cells to GM-CSF and IL-4 and then to MCM prepared by culturing monocytes on immobilized human γ-globulin. Examination of several MCM preparations for the production of cytokines showed that each MCM contained different concentrations of TNF-α, IL-1ß, IL-6 and IFN-α, cytokines known to induce expression of CD83 and p55, two surface molecules that are characteristic of mature DC. However, when combinations of these cytokines were added to immature DC at concentrations comparable to those found in the MCM, they were less effective in maturing DC compared to MCM. These results suggest that additional components are required to affect full maturation of immature DC as well as demonstrating the variability of cytokine generation in different preparations of MCM.

The above-described studies demonstrate that various "conditioned media" are capable of stimulating DC maturation more effectively than addition of purified cytokines but that the "conditioned media" have varying degrees of effectiveness with respect to such stimulation. This is in contrast to the claimed invention, which inheres in the unexpected and surprising finding that the claimed LCM acts as a potent and consistently effective adjuvant to specific antigens when co-administered with the specific antigens in

a mammal by facilitating and enhancing dendritic cell maturation and antigen-presenting cell function in the mammal.

B. Unexpected and Surprising Potency of LCM as Vaccine Adjuvant

The unexpectedly potent adjuvant effect of the claimed LCM when co-administered with various specific antigens has been reported by Applicants in Harris *et al.*, "Products of anti-CD3/anti-CD28 activated lymphocytes induce differentiation and maturation of dendritic cells and have adjuvant-like activity *in vitro* and *in vivo*," *Clinical Immunology*, 2008, In Press.

The paper provides evidence that products of anti-CD3/anti-CD28 activated PBMCs, referred to as LCM, enhances both humoral and cellular responses to vaccines *in vivo* when the LCM is co-administered with a variety of vaccines [hepatitis A (hepA), tetanus and diphtheria toxoid (TDT), rabies or PSA].

To determine the adjuvant activity of LCM *in vivo*, LCM was co-administered with hepA, TDT, rabies or PSA. Animals injected with TDT or HepA vaccine alone exhibited very low to undetectable levels of specific antibodies. By contrast, co-administration of vaccine with LCM resulted in robust production of specific antibodies to tetanus and HepA 7 to 14 days following the first injection in all animals tested, and to diphtheria in two of the three animals. Antibody levels to the vaccines remained present in samples obtained 6 weeks after the initial vaccination. (Page 8, column 2).

In addition, cellular immune responses were assessed in animals vaccinated with TDT, HepA, rabies or PSA by restimulating PBMCs with the respective antigen *in vitro* followed by INFγ assays. PBMCs collected from animals injected with vaccine alone did not produce IFNγ in a recall response. However, PBMCs obtained from animals that received the vaccine in combination with LCM exhibited INFγ responses that were detected in recall assays at day 7 and continued to be present in samples collected over the study period. (Page 8, column 2).

The authors report that the monocytes cultured with LCM differentiated into cells displaying an immature DC phenotype. With further exposure, LCM induced final maturation of immature DC derived from monocytes cultured in GM-CSF and IL-4. (Page 9, column 1).

The authors further report that the classic means of generating human monocyte DC *ex vivo* is to isolate and enrich $CD14^+$ monocytes from peripheral blood and culture them in GM-CSF and IL-4, followed by final maturation with combinations of cytokines such as the "gold standard" consisting of IL-1β, IL-6, TNFα and PGE2 or the "α-type-1 polarizing" containing IL-1β, TNFα, IFNα, IFNγ and poly (I:C). The authors state that recent studies demonstrate that the former cocktail generates DC incapable of secreting the T_H1 cytokine IL-12 and suggest that the latter produces strongly adherent DC difficult to recover from culture, rendering both methods problematic in the development of DC-based therapies. In contrast, DC generated from monocyte precursors cultured in LCM are capable of generating immune responses to primary and recall antigens. This allows for the use of LCM in large scale production of DC for immunotherapeutic purposes. (Page 9, bottom column 1 to top of column 2).

The authors state that, to their knowledge, this is the first report that documents *in vitro* and *in vivo* adjuvant activity of LCM regardless of the method used to generate cytokines and chemokines from cultured human PBMCs, monocytes or T cells. They point out that the *in vitro* functional studies clearly demonstrate the ability of LCM to augment T cell responses and the *in vivo* studies show that LCM augments cellular and humoral responses to vaccines, which indicates that LCM acts to enhance both T_H1 and T_H2 responses. (Page 10, column 1).

C. Unpredictability of Conventional Vaccine Adjuvants

In contrast to the claimed invention, which provides the unexpected and surprising finding that the claimed LCM acts as a potent and consistently effective adjuvant for a variety of specific antigens when co-administered with the specific antigens, it has been known for many years by those skilled in the art

that conventional adjuvants typically are unpredictable in their ability to enhance specific immune responses to a specific antigen, i.e. vaccine.

For example, Edelman ("An update on vaccine adjuvants in clinical trial," *Aids Research and Human Retroviruses*, 8 (8):1409-1411, 1992) teaches that:

> Despite striking immunological advances in the past 20 years, **adjuvant use remains largely empiric**. Their mechanisms of action in vitro and in vivo are varied as the immune response itself. In fact, few if any studies have revealed how any adjuvant operates in vivo. **The unpredictability of adjuvant effects results from a complex interplay between route of administration, timing of innoculations, antigen dose, antigen construct, adjuvant formulation, host species, and within-species genetic variation**. As a consequence of these variables, **antigens are best matched with adjuvants by means of a trial by error process of iterative experiments**. Page 1409, 7th paragraph. [Emphasis added].

Additionally, in a Guideline prepared by the Committee For Proprietary Medicinal Products (published by The European Agency for the Evaluation of Medicinal Products-Evaluation of Medicines for Human Use, London, March 25, 2004), it is stated that:

> Adjuvant activity is a result of multiple factors and **an enhanced immune response obtained with one antigen cannot as a rule be extrapolated to another antigen**. Page 4, 6th paragraph.

It also is stated (echoing Edelman from twenty-two years prior):

> **The unpredictability of adjuvant effects in humans results from a complex interplay between such factors as route of administration, antigen dose and the nature of the antigen.** Page 5, 4th paragraph.

Summary

The Examiner takes the position that the claimed invention is obvious over Meidenbauer in view of Baxevanis and Quinn. But, even if one skilled in the art would be motivated to combine the teachings of Meidenbauer with Baxevanis and Quinn, there would not be a reasonable expectation of success to achieve the claimed invention, which provides the unexpected and surprising finding that the claimed LCM acts as a potent, efficient and consistently effective adjuvant by to a specific antigen when co-

administered with the specific antigen by facilitating and enhancing dendritic cell maturation and antigen-presenting cell function, because Baxevanis solely teaches generic T cell stimulation and activation of pre-existing T cells using supernatants derived from CD3-stimulated lymphocytes, and does not disclose or even suggest that their supernatant acts as a potent and effective adjuvant to facilitate and enhance an antigen-specific response when co-administered with a specific antigen. In addition, there would not be a reasonable expectation of success because one skilled in the art would appreciate that adjuvants generally are unpredictable and not very effective to enhance an immune response to a specific antigen. Thus, because Baxevanis solely teaches generic T cell stimulation and activation of pre-existing T cells using supernatants derived from CD3-stimulated lymphocytes, and does not disclose or even suggest that their supernatant will facilitate and enhance an antigen-specific response when co-administered with a specific antigen, one skilled in the art would not be motivated to combine the teachings of Meidenbauer with Baxevanis and Quinn and have a reasonable expectation of success of achieving the claimed invention.

Applicants believe that fees for a three-month extension of time are due with this filing. Such fee is being simultaneously paid via electronic funds transfer with this submission. The Commissioner is hereby authorized to charge any additional fees required or to credit any overpayment to Deposit Account 20-0809. The applicant(s) hereby authorizes the Commissioner under 37 C.F.R. §1.136(a)(3) to treat any paper that is filed in this application which requires an extension of time as incorporating a request for such an extension.

Respectfully submitted,

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